Multiple-Breath Washout Outcomes Are Sensitive to Inflammation and Infection in Children with Cystic Fibrosis

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Abstract

Rationale: The lung clearance index is a measure of ventilation distribution derived from the multiple-breath washout technique. The lung clearance index is increased in the presence of lower respiratory tract inflammation and infection in infants with cystic fibrosis; however, the associations during the preschool years are unknown.

Objectives: We assessed the ability of the lung clearance index to detect the presence and extent of lower respiratory tract inflammation and infection in preschool children with cystic fibrosis.

Methods: Ventilation distribution outcomes were assessed at 82 visits with 58 children with cystic fibrosis and at 38 visits with 31 healthy children aged 3–6 years. Children with cystic fibrosis also underwent bronchoalveolar lavage fluid collection for detection of lower respiratory tract inflammation and infection. Associations between multiple-breath washout indices and the presence and extent of airway inflammation and infection were assessed using linear mixed effects models.

Results: Lung clearance index was elevated in children with cystic fibrosis (mean [SD], 8.00 [1.45]) compared with healthy control subjects (6.67 [0.56]). In cystic fibrosis, the lung clearance index was elevated in individuals with lower respiratory tract infections (difference compared with uninfected [95% confidence interval], 0.62 [0.06, 1.18]) and correlated with the extent of airway inflammation.

Conclusions: These data suggest that the lung clearance index may be a useful surveillance tool for monitoring the presence and extent of lower airway inflammation and infection in preschool children with cystic fibrosis.

Keywords: multiple-breath washout; child; inflammation; infection; cystic fibrosis

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Pulmonary inflammation and lower respiratory tract infections are present in the first months of life in children with cystic fibrosis (CF), often in the absence of respiratory symptoms (1). The presence of inflammatory markers (neutrophils, neutrophil elastase [NE], IL-8) and pathogens (including *Pseudomonas aeruginosa* and *Staphylococcus aureus*) in the lower respiratory tract in early life is associated with the development and progression of structural lung disease and long-term impairments in lung function in children with CF (2–4). Early surveillance of lung disease is essential to identifying and treating respiratory infections, minimizing pulmonary exacerbations, and delaying the onset of structural lung damage and lung function decline.

The lung clearance index (LCI) is a measure of ventilation distribution derived from the multiple-breath washout (MBW) technique. In preschool children with CF, MBW is easier to perform and more often abnormal than spirometry or plethysmography (5). An abnormal LCI during the preschool years is predictive of abnormal LCI and spirometry outcomes at school age (6). In a longitudinal study of patients with CF aged 6–20 years, LCI was the first index of lung disease to decline and the strongest predictor of lung disease progression (7). In addition, LCI has been shown to respond to therapeutic interventions and predict pulmonary exacerbations in children with CF (8–10). In the era of newborn screening, LCI has the potential to be an important clinical tool for the monitoring of early, often mild CF lung disease.

Limited data exist on the ability of MBW to predict the presence of pulmonary inflammation and infection. During infancy, LCI correlates with neutrophil count, IL-8, and pathogen density in bronchoalveolar lavage (BAL) fluid (11, 12). In preschool children, LCI is higher in those with *P. aeruginosa* detected in the upper airway (5), but there have been no studies correlating LCI and lower respiratory tract inflammation or infections in this age group. The presence of bronchiectasis increases dramatically between the ages of 3 and 6 years in children with CF; thus, this period represents a significant window of opportunity for intervention (4). The aim of this study was to assess the ability of the LCI and other markers of ventilation distribution to detect the presence and extent of lower respiratory tract inflammation and infection in preschool children with CF. Some of the results of these studies were previously reported in the form of abstracts (13, 14).

Methods

Study Population

This analysis took place in the setting of an ongoing longitudinal study between the Perth and Melbourne centers of the Australian Respiratory Early Surveillance Team for Cystic Fibrosis (AREST CF) as well as Riley Hospital for Children in Indianapolis, Indiana. Children with CF were diagnosed following newborn screening and were enrolled in the AREST CF early surveillance program at Princess Margaret Hospital in Perth, Australia, or Royal Children's Hospital in Melbourne, Australia (1). This program starts at diagnosis (approximately age 3 mo) and involves annual age-appropriate lung function testing 2–4 days prior to performing chest computed tomography and BAL until the age of 6 years. Healthy control subjects with no history of respiratory disease, preterm birth, or current respiratory symptoms or medications were also recruited to perform MBW testing only from the communities in Perth and Melbourne in Australia and Indianapolis, Indiana, in the United States. The ethics committee of each institution approved the study, and parents consented to each aspect of the study separately.

Table 1. Demographics of study population

	Cystic Fibrosis	Healthy Control Subjects
Subjects, n	58	31
Visits, n	82	38
Age, yr	5.1 ± 1.0	5.0 ± 1.0
Sex, male	36 (44%)	18 (47%)
Height *z*-score	−0.04 ± 0.98	0.23 ± 1.24
Weight *z*-score	0.35 ± 0.86	0.67 ± 1.45
BMI *z*-score	0.55 ± 0.87	0.77 ± 1.44
FRC, L	0.89 ± 0.22	0.92 ± 0.18
Lung clearance index	8.00 ± 1.45*	6.67 ± 0.56
First-moment ratio, M_1/M_0	1.73 ± 0.30*	1.52 ± 0.12
Second-moment ratio, M_2/M_0	6.48 ± 2.44*	4.67 ± 0.79
Current respiratory symptoms	32 of 82 (39%)	
Phe508del/Phe508del	35 of 82 (43%)	
Phe508del/other	72 of 82 (88%)	
Total cell count, $\times 10^5$/ml	8.13 ± 14.9	
Neutrophils, %	23.6 ± 22.5	
Neutrophil elastase present	22 of 81 (27%)	
Neutrophil elastase, ng/ml	1,014 ± 2,314	
IL-8, pg/ml	3,557 ± 8,252	
Respiratory infection	32 of 81 (39%)	
Nonproinflammatory pathogens	4 of 81 (5%)	
Proinflammatory pathogens	28 of 81 (35%)	
More than one proinflammatory pathogen	13 of 81 (16%)	
Staphylococcus aureus	10 of 81 (12%)	
Haemophilus influenzae	9 of 81 (11%)	
Pseudomonas aeruginosa	8 of 81 (10%)	
Aspergillus spp.	8 of 81 (10%)	
Streptococcus pneumoniae	5 of 81 (6%)	

Definition of abbreviations: BMI = body mass index; M1/M0 = first-moment ratio; M2/M0 = second-moment ratio.

Data are presented as mean ± SD or n (%) unless otherwise stated. Height, weight, and BMI *z*-scores were calculated using World Health Organization growth standards (23, 24). Current respiratory symptoms were defined as either parent- or clinician-reported symptoms in the last month. Respiratory infection was defined as pathogen density greater than 10^4 cfu/ml, excluding mixed oral flora.

*$P < 0.05$.

Multiple-Breath Washout

MBW was performed using 100% oxygen to wash out resident nitrogen from the lungs (EXHALYZER D and SPIROWARE 3.1; ECO MEDICS AG, Dürnten, Switzerland) using a silicone mouthpiece (15, 16). FRC, LCI, first-moment ratio (M_1/M_0), and second-moment ratio (M_2/M_0) were derived from visits with at least two acceptable measurements with no evidence of leak or irregular breathing pattern, in line with current international standards (17). Two independent, experienced assessors (K.A.R. and R.E.F.) performed data quality control using a systematic approach based on current consensus statement guidelines (18).

Bronchoalveolar Lavage

A bronchoscopy during which BAL fluid was collected was performed within 3 days of MBW testing. The right middle lobe was lavaged with three aliquots of saline (1 ml/kg body weight), and an additional aliquot was lavaged into the lingula or the most affected lobe identified by computed tomography (1, 19). The first aliquot of BAL fluid was processed for the detection of bacteria, viruses, and fungi using standard culture techniques. Subsequent aliquots were pooled for the quantification of pulmonary inflammation, which included total and differential cell counts as well as measurement of free NE activity (lower limit of detection, 200 ng/ml) (1). An inflammatory response score was created by taking a weighted average of the logged BAL responses: log total cell count per milliliter of fluid retrieved, log neutrophils per milliliter of fluid retrieved, log NE, and log IL-8. The weights of each component were derived using principal component analysis. Pulmonary infection was defined as colony counts for a specific organism (excluding mixed oral flora) greater than 10^4 cfu/ml. Bacterial pathogens known to elicit a proinflammatory response when isolated from the lower respiratory tract (*P. aeruginosa*, *S. aureus*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Aspergillus* spp.) were described as proinflammatory pathogens (19).

Respiratory Symptoms

A standardized respiratory symptoms questionnaire was administered on the morning of the BAL of children with CF to gain information on parentally reported symptoms (cough, upper respiratory tract infection, sputum production) and current medication use. A respiratory clinician also performed a physical examination of the children and reported the presence or absence of wheeze, crackles, or respiratory tract infection. Children were classified as symptomatic if any symptoms were reported within the last month.

Statistical Analysis

The upper limits of normal for MBW indices were calculated from our prospective healthy control population of children, a subset of which was previously reported (15). Associations between MBW indices and the extent of inflammation and infection were assessed using linear mixed effects models with random intercepts for repeated visits to account for clustering. All models were adjusted for age at visit and study center. The coefficients of the fixed effects of the linear mixed models are presented with 95% confidence intervals and *P* values. These coefficients represent the adjusted differences between case and control groups. For example, children with a respiratory infection had an adjusted LCI that was 0.62 greater than that of the children without an infection. The reference group for all the respiratory infection subgroups is individuals with no respiratory infection. Sensitivity and specificity values were calculated. All analyses were performed using Stata 13.0 software (StataCorp, College Station, TX).

Table 2. Effect of pulmonary inflammation, infection, and clinical characteristics on measures of ventilation distribution in preschool children with cystic fibrosis

	LCI	M_1/M_0	M_2/M_0
Clinical characteristics			
Respiratory symptoms	−0.25 (−0.75, 0.25)	0.03 (−0.08, 0.13)	−0.24 (−1.06, 0.59)
Severe genotype	0.18 (−1.70, 2.06)	0.16 (−0.24, 0.56)	0.29 (−2.93, 3.52)
Pulmonary inflammation			
Total cell counts	**0.25 (0.04, 0.46)***	**0.07 (0.03, 0.11)†**	**0.45 (0.11, 0.79)***
Neutrophil cells	0.10 (−0.04, 0.25)	**0.03 (0.01, 0.06)***	0.21 (−0.03, 0.44)
Presence of neutrophil elastase	0.58 (−0.05, 1.22)	**0.15 (0.04, 0.30)†**	**1.13 (0.10, 2.16)***
Neutrophil elastase concentration	0.05 (−0.08, 0.17)	0.02 (−0.01, 0.05)	0.12 (−0.08, 0.32)
Inflammatory response score	**0.28 (0.07, 0.49)†**	**0.07 (0.02, 0.11) †**	**0.47 (0.11, 0.83)***
Respiratory infection	**0.62 (0.06, 1.18)***	**0.11 (0.01, 0.23)***	0.78 (−0.13, 1.70)
Nonproinflammatory pathogen	0.53 (−0.48, 1.55)	0.18 (−0.04, 0.40)	0.78 (−0.79, 2.34)
Proinflammatory pathogen	**0.65 (0.10, 1.20)***	0.11 (−0.01, 0.22)	0.80 (−0.08, 1.69)
More than one proinflammatory pathogen	**1.04 (0.40, 1.68)†**	**0.18 (0.06, 0.31)†**	**1.45 (0.45, 2.45)†**
Staphylococcus aureus	−0.03 (−1.11, 1.06)	0.20 (−0.01, 0.41)	1.11 (−0.59, 2.82)
Haemophilus influenzae	0.23 (−0.55, 1.01)	0.02 (−0.13,0.18)	−0.01 (−1.22, 1.21)
Pseudomonas aeruginosa	**0.93 (0.16, 1.71)***	0.07 (−0.10, 0.23)	0.69 (−0.59, 1.96)
Aspergillus spp.	0.46 (−0.41, 1.34)	0.06 (−0.11, 0.24)	0.37 (−1.01, 1.75)
Streptococcus pneumoniae	0.71 (−0.35, 1.78)	0.16 (−0.05, 0.38)	1.24 (−0.47, 2.96)

Definition of abbreviations: LCI = lung clearance index; M_1/M_0 = first-moment ratio; M_2/M_0 = second-moment ratio.

Data are presented as mixed model coefficients adjusted for repeated measures and testing center (95% confidence interval). These coefficients represent the adjusted differences between case and control groups. For example, children with a respiratory infection had an adjusted LCI that was 0.62 greater than that of the children without an infection. The reference group for all the respiratory infection subgroups is individuals with no respiratory infection. Respiratory infection was defined as pathogen density greater than 10^4 cfu/ml, excluding mixed oral flora. Proinflammatory pathogens are pathogens known to elicit a proinflammatory response when isolated from the lower respiratory tract (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Aspergillus* spp.) (19). The inflammatory response score was created by taking a weighted average of the logged inflammatory responses: log total cell count per milliliter of fluid retrieved, log neutrophils per milliliter of fluid retrieved, log neutrophil elastase, and log IL-8. Bold typeface indicates significant associations with $P < 0.05$.

*$P < 0.05$.

†$P < 0.01$.

Results

Study Population

Paired ventilation distribution data from acceptable MBW tests and inflammation and infection data from BAL fluid were available for 82 visits with 58 children with CF. In addition, MBW data was available for 38 visits with 31 healthy control subjects between 3 and 6 years of age. During the study period (January 2013 to December 2015), we measured MBW in children with CF during 104 annual review BAL visits and in healthy control children at 51 test visits. Of the 104 test visits of children with CF, 82 had at least two acceptable MBW trials (79% feasibility), and of these visits, 56 had at least three acceptable MBW trials (68%). Of the 51 test visits with healthy control subjects, 38 had at least two acceptable MBW trials (75% feasibility), and of these visits, 25 had at least three acceptable trials (66%). In this analysis, 24 children with CF and 7 healthy control subjects contributed two test visits measured approximately 12 months apart (11.6 ± 2.0 mo).

Upper limits of normal for LCI (7.71), M_1/M_0 (1.70), and M_2/M_0 (5.53) were calculated on the basis of data derived from our healthy control population. There were no differences in age, height, weight, or FRC between healthy control subjects and children with CF ($P > 0.05$) (Table 1). LCI, M_1/M_0, and M_2/M_0 were significantly higher in children with CF than in healthy control subjects (Table 1). We found that 55% of preschool children with CF had an abnormal LCI, 52% had an abnormal M_1/M_0, and 64% had an abnormal M_2/M_0.

Of the 58 children with CF, 88% were heterozygous for the Phe508del mutation (Table 1). In the 82 annual surveillance visits with children with CF, respiratory symptoms were reported in 39% of visits; and in BAL fluid samples, NE was detected in 27%, a respiratory pathogen was detected in 39%, and a proinflammatory pathogen was detected in 35%. The most common proinflammatory pathogen detected was *S. aureus* (12%), followed by *H. influenzae* (11%), *P. aeruginosa* (10%), *Aspergillus* spp. (10%), and *S. pneumoniae* (6%).

Clinical Status

There were no differences in MBW outcomes between individuals with CF with and without current respiratory symptoms. In addition, there were no differences between individuals with mild or severe CF genotypes (Table 2).

Pulmonary Inflammation

MBW outcomes significantly correlated with the extent of inflammation in the lower respiratory tract. LCI, M_1/M_0, and M_2/M_0 were positively associated with the total number of inflammatory cells and the mean cumulative inflammatory response score (Table 2). In addition, M_1/M_0, but not LCI or M_2/M_0, was significantly associated with the percentage of neutrophils (Figure 1). Both moment ratios, but not LCI, were significantly higher in children with CF with detectable free NE activity than in those without detectable free NE. None of the MBW indices correlated with the concentration of NE in the BAL fluid. MBW outcomes had low sensitivity (38 to 44%) but high specificity (85 to 88%) to detect free NE in the BAL (Table 3).

Respiratory Infection

LCI and M_1/M_0, but not M_2/M_0, were higher in those with a lower respiratory tract infection than in those with no detectable infection (Table 2). LCI was higher in individuals with a proinflammatory pathogen detected, but it was not higher in individuals with a nonproinflammatory pathogen (Figure 2). All MBW indices were significantly higher in individuals with more than one proinflammatory pathogen detected in the lower respiratory tract. When the proinflammatory pathogens were examined individually, LCI was significantly higher during visits when *P. aeruginosa* was detected in the BAL than in those who were not infected. There were no significant

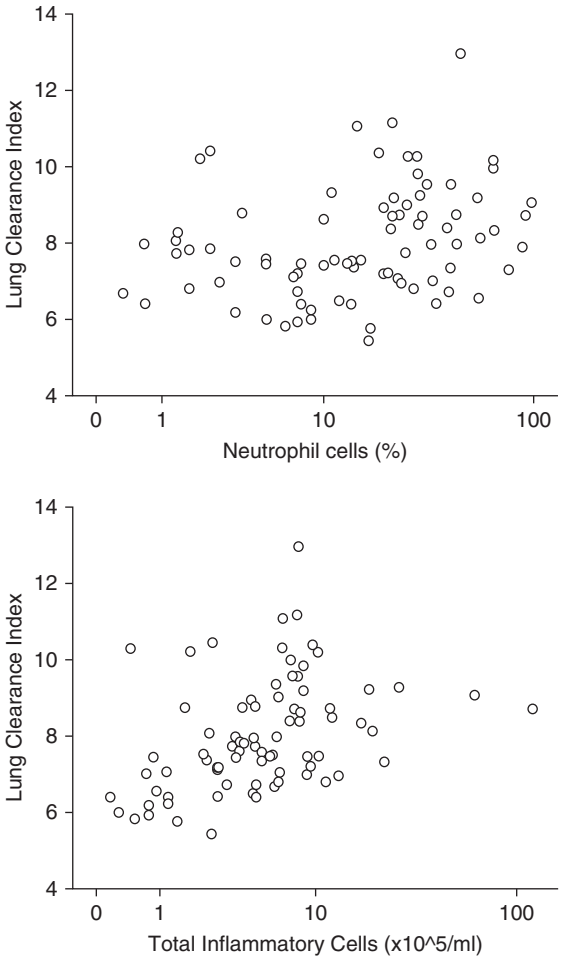

Figure 1. Association between lung clearance index and the extent of neutrophils (*top graph*) and total inflammatory cells (*bottom graph*) in the lower respiratory tract.

Table 3. Ability of measures of ventilation distribution to detect the presence of neutrophil elastase and respiratory infections in preschool children with cystic fibrosis

	LCI	M_1/M_0	M_2/M_0
Neutrophil elastase			
Sensitivity	41 (26, 57)	44 (29, 60)	38 (25, 54)
Specificity	85 (70, 94)	88 (74, 96)	86 (70, 95)
Respiratory infection			
Sensitivity	48 (32, 64)	49 (33, 65)	45 (30, 60)
Specificity	73 (56, 85)	73 (57, 86)	71 (54, 85)

Definition of abbreviations: LCI = lung clearance index; M_1/M_0 = first-moment ratio; M_2/M_0 = second-moment ratio.
Data are presented as percentage (95% confidence interval). Upper limit of normal for LCI = 7.71, M_1/M_0 = 1.70, and M_2/M_0 = 5.53. Respiratory infection was defined as pathogen density greater than 10^4 cfu/ml, excluding mixed oral flora.

correlations between MBW outcomes and the presence of other individual pathogens in the BAL. MBW outcomes had low sensitivity (45 to 49%) but moderate specificity (71 to 73%) to detect a significant respiratory infection (Table 3).

Discussion

The objective of this study was to investigate whether outcomes of ventilation distribution measured using the MBW test were elevated in the presence of lower respiratory tract inflammation and infection in preschool children with CF. We report greater ventilation inhomogeneity in individuals with CF who had higher inflammatory cell counts and free NE detected in the BAL fluid. Children with detectable pathogens known to elicit a proinflammatory response in the lung had higher LCI and moment ratio values than uninfected children, and *P. aeruginosa* infection was individually associated with higher LCI values. These data suggest that the LCI and moment ratios derived by use of the MBW technique may be important surveillance tools for monitoring clinical status in preschool children with CF. Furthermore, these indices could serve as important outcome measures for clinical trials of therapies that treat pulmonary infection and inflammation in young children with CF.

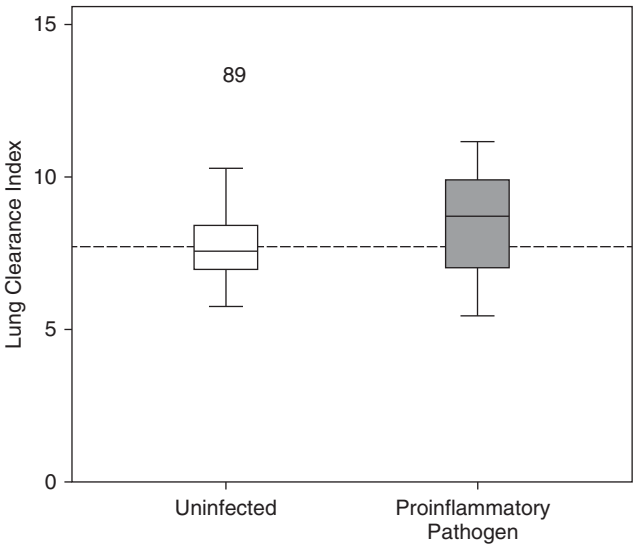

Figure 2. Lung clearance index in children with cystic fibrosis with and without a lower respiratory tract infection. Respiratory infection is defined as pathogen density greater than 10^4 cfu/ml, excluding mixed oral flora. Proinflammatory pathogens are pathogens known to elicit a proinflammatory response when isolated from the lower respiratory tract (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Aspergillus* spp.) (19). *Dotted horizontal line* represents the upper limit of normal for lung clearance index = 7.71.

Limited data exist on the ability of the MBW technique to predict the presence of airway inflammation. LCI correlates with neutrophil and IL-8 concentrations in BAL fluid in infants with CF (11). However, longitudinally, there were no associations between markers of lower respiratory tract inflammation, such as NE and IL-8 levels, and MBW outcomes over the first 2 years of life (12). To our knowledge, this is the first study to assess associations between pulmonary inflammation and MBW outcomes in preschool children with CF. We report significant associations between LCI and the total number of inflammatory cells detected in the BAL. LCI was significantly associated with the inflammatory response score, which incorporates the primary inflammatory markers, including total cells, neutrophils, IL-8, and NE. In addition, the moment ratios were significantly associated with total inflammatory cells, neutrophil cells, and presence of NE. Moment ratios from the MBW technique describe the degree of skewness of the nitrogen washout curve. Increased moment ratios are thought to represent an increased release of inert gas toward the end of the washout and therefore may correlate more closely with markers of inflammation or infection in the small peripheral airways of the lung (20). Neutrophil-dominated pulmonary inflammation in young children with CF is one of the earliest signs of lung disease and is associated with the development and progression of structural lung damage, including bronchial wall thickening and bronchiectasis (2). The prevalence of structural abnormalities detected via imaging modalities increases rapidly during the early preschool years, and the extent of structural lung disease is significantly associated with increased LCI and moment ratios in children with CF (15, 21). The MBW technique may be an important tool to monitor the development of early ventilation defects that may be caused by inflammation in the lower airways, and it may be an important endpoint in therapies designed to prevent early structural damage.

In this analysis, MBW outcomes were elevated in young children with a lower

respiratory tract infection. It was previously shown that LCI is elevated in young children with CF who had *P. aeruginosa* detected on oropharyngeal swabs in the 6 months prior to MBW testing (5). LCI was higher in infants with CF who had *P. aeruginosa* detected in BAL fluid (11). In addition, LCI values were higher in children and young adults with CF who had chronic *P. aeruginosa* infection persistently detected within the last 6 months (21). In a recent longitudinal study of 156 children with CF, researchers reported that children with at least one positive oral throat swab culture with *S. aureus* had higher LCI values than those without *S. aureus* (22). We report that LCI is almost 1 unit higher in preschool children with CF with detectable *P. aeruginosa* in BAL fluid at the time of MBW testing than in uninfected children with CF. The presence of any proinflammatory pathogen known to elicit a proinflammatory response in the lower respiratory tract was also associated with higher LCI values, and detection of more than one of these pathogens in the BAL was associated with higher LCI and moment ratios. Respiratory infections with proinflammatory pathogens are likely to cause narrowing, closure, and/or mucus obstruction in the airways, resulting in uneven ventilation distribution throughout the lung and thus higher LCI values.

A previous study by our group revealed that infection with a proinflammatory pathogen resulted in accelerated lung function decline (increased ventilation inhomogeneity) during the first 2 years of life compared with infants who remained uninfected (12). Longitudinal studies of preschool children are required to determine whether transient increases in LCI above the child's individual baseline is indicative of the presence of a new pulmonary infection.

Strengths of the present study are the use of BAL fluid for the assessment of lower respiratory tract inflammation and infection and that clinical BAL data and MBW outcomes were assessed within the same week. Furthermore, we prospectively recruited healthy children from the local community to provide control reference MBW data. Limitations of the study are that children with CF underwent annual surveillance, so we do not have access to lower airway inflammation and infection data in the time between annual visits. In addition, children with CF in this study were deemed well enough to receive a general anesthetic and were clinically stable at the time of the MBW testing. Therefore, we are unable to comment on whether MBW outcomes were altered in individuals with moderate or severe respiratory symptoms. We also do not yet have sufficient numbers in our cohort to perform longitudinal analyses of the changes in MBW over time with clinical status.

Our findings support the notion that the MBW technique is an important surveillance tool in the monitoring of early lung disease in young children with CF. Global measures of ventilation distribution were associated with the presence and extent of pulmonary inflammation and infection in the lower respiratory tract at the time of MBW testing. These data provide further evidence that outcomes obtained with the MBW technique can reflect mild, heterogeneous, early changes in the lung caused by the development of early CF lung disease. Further longitudinal studies are required to confirm these results and determine the ability of MBW outcomes to detect changes in disease status in early life. Our results support further development and validation of the MBW technique to serve as an important noninvasive tool for monitoring early manifestations of CF lung disease as well as an endpoint in therapeutic trials aimed at reducing the development of irreversible structural disease in young children with CF. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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